



Review

# The nonmutagenic repair of broken replication forks via recombination

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## Abstract

When replication forks stall or collapse at sites of DNA damage, there are two avenues for fork rescue. Mutagenic translesion synthesis by a special class of DNA polymerases can move a fork past the damage, but can leave behind mutations. The alternative nonmutagenic pathways for fork repair involve cellular recombination systems. In bacteria, nonmutagenic repair of replication forks may occur as often as once per cell per generation, and is the favored path for fork restoration under normal growth conditions. Replication fork repair is almost certainly the major function of bacterial recombination systems, and was probably the impetus for the evolution of recombination systems. Increasingly, the nonmutagenic repair of replication forks is seen as a major function of eukaryotic recombination systems as well.

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## 1. Introduction

Replication forks are elaborate protein machines, evolutionarily engineered for speed and fidelity. These characteristics come at the expense of limited tolerance for structural aberrations in the DNA template. Thus, collisions with DNA lesions or strand breaks can result in the arrest or collapse of the replication fork. These events appear to occur often in most cells, and they set into motion pathways for replication fork repair. Restoring a replication fork can involve translesion DNA synthesis (TLS) by a special class of DNA polymerases now known to be ubiquitous in nature [1–5]. However, these enzymes lack the fidelity of the normal replicative polymerases, and TLS results in an increase in mutation frequency. In addition, DNA

strand breaks and some other kinds of DNA lesions are not readily bypassed by TLS. A second, nonmutagenic set of pathways for replication fork repair exists, collectively called recombinational DNA repair. Recognition that recombination represented a major pathway for the repair of replication forks has been documented in a series of recent reviews [6–14].

The homologous recombination systems of every cell are in one sense the molecular engines that enable much of the science of genetics, and for decades a perceived role in the generation of genetic diversity provided an adequate *raison d'être* for their study. However, it was clear to many researchers that a need for genetic diversity did not provide a rationale sufficient to explain the evolution of complex recombination systems [15], and some prescient connections between recombination and replication forks were proposed quite early [16–18]. More recent insights [7,9,14,19] have paved the way to a paradigm

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shift in the study of recombination, focusing now on what is clearly a major DNA repair process in all cells. In brief, replication forks collapse or arrest at sites of DNA damage often, and the consequences of these events are catastrophic if not repaired. Thus, the recombination systems needed to repair replication forks are essential cellular components. The requirement for replication fork repair provides a compelling rationale for the evolution of recombination systems and their ubiquitous maintenance in all cells. In bacteria, the repair of replication forks is the central function of homologous recombination systems.

As described in more detail below, the pathways for nonmutagenic recombinational DNA repair of replication forks are complex, but all encompass the following steps: first a replication fork encounters a structural barrier and replication ceases, then a recombination system takes over and reconstitutes a viable replication fork structure, and finally a specialized system for replication restart loads the required enzymes onto the DNA and allows replication to proceed. A replication fork which encounters a DNA strand break in one of the template strands gives rise to complete separation of one branch of the fork (a double strand break (DSB)), and is said to be collapsed. A fork that has halted at the site of an impassable lesion or other barrier is said to be arrested. There are four important concepts that must be introduced prior to a detailed discussion of the systems that address these situations.

### *1.1. Homologous genetic recombination and DNA strand invasion*

Many of the insights garnered from work on conjugational recombination in bacteria and meiotic recombination in yeast and other eukaryotes came together elegantly in the 1983 double strand break repair model for meiotic recombination [20] (Fig. 1). This pathway, which with minor modifications is now well-established as the major nonmutagenic repair process for double strand breaks in many contexts [21]. A centerpiece of this pathway is a DNA strand invasion step, a type of reaction first suggested by Meselson and Weigle in 1961 [22]. In its modern iteration, a double strand end is processed to create a 3' extension. This single stranded region is then bound by a recombinase and paired with its complement within a duplex. This is the strand invasion step,

resulting in displacement of the original complementary strand, creating an intermediate often referred to as a D-loop. The use of a 3'-ending strand for strand invasion allows that 3' end to be used as a primer for subsequent replication steps. Strand invasion lies at the heart of a wide range of recombination reactions.

### *1.2. Recombination-dependent replication*

The process of DNA strand invasion leads directly to a new pathway for the initiation of DNA replication. Initiation of replication at a replication origin requires proteins to separate the DNA strands and to load the replicative DNA helicase. The alternative process is origin-independent, and is called recombination-dependent replication (RDR). DNA strand separation is effectively brought about by the recombinase-mediated strand invasion step (Fig. 2). The subsequent loading of the replicative helicase and the reconstitution of the entire replication fork in bacteria depends on a specialized set of proteins called the restart primosome [12]. RDR is a required step in the replication of bacteriophage T4 DNA [23], and studies of that system have provided much insight into mechanisms. Extensive evidence for the existence of this alternative path of replication initiation in bacteria was provided by Kogoma [19]. The process is also manifest in eukaryotes in processes such as break-induced replication [24,25]. The bacterial and eukaryotic observations of origin-independent replication must be considered a manifestation of the cellular systems set up to reinitiate replication after recombinational DNA repair of a broken fork. Skalka provided the first suggestion that RDR (prior to establishment of the acronym) might be used to repair a replication fork collapsed by virtue of an encounter with a template strand break [17]. Her proposal made use of recombination mechanisms proposed by Radding just a few years prior [26].

### *1.3. Replication fork regression*

The concept of branch migration was introduced in 1970 by Davidson and coworkers [27]. A somewhat elaborate form of branch migration can occur at an arrested replication fork, as first proposed by Higgins et al. [28]. The fork can be migrated backwards so as to re-anneal the original template strands, and extrude

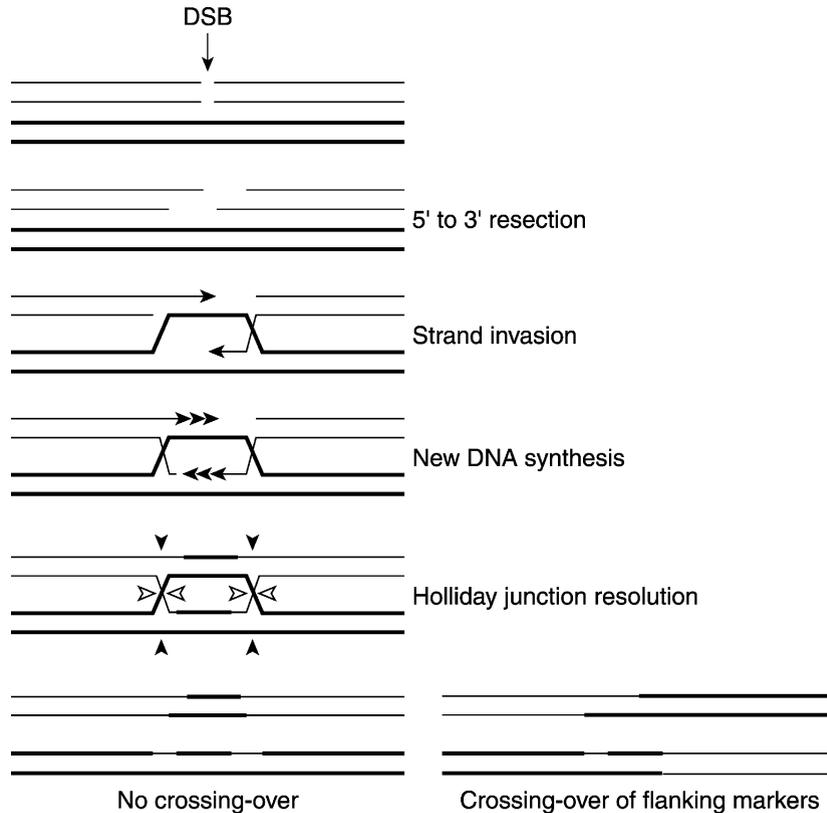


Fig. 1. The double strand break repair model for homologous genetic recombination. The initiating event is the double strand break (DSB). The free DNA ends are processed, and 3' ends are used to invade a homologous duplex DNA. Thick and thin lines are used to allow the fates of DNA derived from each chromosome to be traced. Arrowheads at the end of DNA lines denote 3' ends. Free arrowheads in the Holliday structure resolution step denote potential DNA cleavage sites, and the open and closed arrows represent sets of cleavages that can generate the viable recombinants shown at bottom. For example, cleavage at the open arrows would generate the "no crossing-over" product shown at left.

the newly synthesized DNA strands as a short branch (Fig. 3). This process is called fork regression. Once the regression has moved beyond any gaps that may exist at the arrested fork, the product is a four-way junction called a Holliday structure, a classic recombination intermediate. However, the usual manner of its depiction suggested to Cozzarelli and coworkers that the structure be dubbed a chicken foot [29], and this more colorful label has stuck for Holliday structures generated by the fork regression process. Fork regression offers some avenues for fork repair that do not involve double strand breaks or DNA strand invasion steps, as detailed later. Once a fork has regressed, branch migration can take it back in the opposite direction to regenerate the three-way junction at the fork.

#### 1.4. Replication forks stall often

Under normal growth conditions, bacteria may suffer the demise of a replication fork at a frequency approaching once per cell per generation. In terms of frequency-of-use, the nonmutagenic repair of stalled replication forks is thus the most important function of the bacterial homologous genetic recombination systems [8,30–32]. Conjugation and transduction, which also involve recombination, are normally very rare processes.

This conclusion is based primarily on an analysis of the effects of mutations that eliminate key components of the repair pathways. Recombination to repair a replication fork can result in a crossover behind

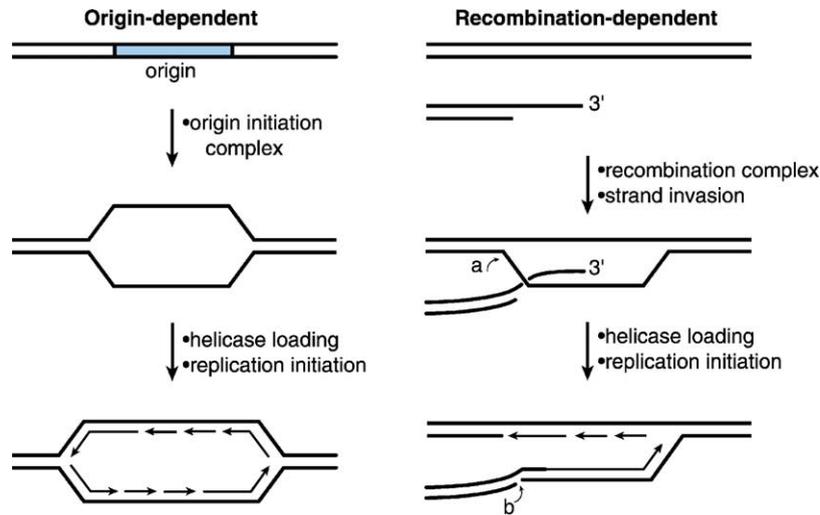


Fig. 2. Two mechanisms for the initiation of replication. Origin-dependent replication initiation is shown at left, and recombination-dependent replication (RDR) is shown at right. In the latter pathway, the letters a and b denote sites of hypothetical cleavage and ligation events, respectively, that are included to illustrate one pathway by which a D-loop generated via strand invasion can be converted into a viable replication fork.

the restored replication fork, leading eventually to the creation of a contiguous chromosomal dimer that cannot segregate into daughter cells. These dimers are resolved to monomers by the *Escherichia coli* XerCD site-specific recombination system. In mutant strains lacking XerCD, about 15% of the cells have chromosomal dimers and cannot complete normal cell division [8,33–35], a result which indicates that a lower limit of at least 15% of the cells in the population undergo recombination events triggered by replication under normal growth. Recent work has shown that most recombinational repair events proceed so as to bias the outcome against the generation of crossovers and prevent the generation of dimers [36], suggesting that many more than 15% of the cells in a population undergo recombinational repair of replication forks under normal growth.

The highest estimates have come from study of mutations that affect the function of the restart primo-

some, originally called the  $\phi$ X174-type primosome. There are seven proteins in this complex, and the key protein in the assembly of the complex is the PriA protein. Isolated in early studies of in vitro replication using  $\phi$ X174 DNA templates, the observation that this primosome was not needed for replication initiation at *oriC* [37,38] came as a surprise. Nevertheless, null mutants of *priA* later proved to be only marginally viable, suggesting an important cellular function [39–41]. The *priA* mutants also induced the SOS response under normal growth conditions [40]. Building on a demonstration that SOS was induced only in cells with active replication forks [42], and earlier suggestions [43], Marians and coworkers reasoned that if PriA was not needed at *oriC*, perhaps replication forks were stalling at DNA damage. PriA and the other primosome proteins might then be necessary to get replication going again. Most important, to explain the marginal viability of the *priA* mutants,

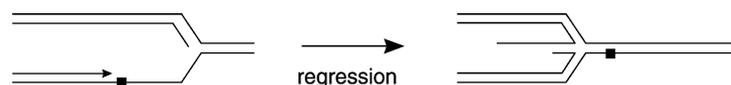


Fig. 3. Regression of a replication fork. Regression is a reverse branch migration in which the original template strands are repaired, and the newly synthesized strands are annealed as the replication fork is forced backwards. The product structure at right is a Holliday junction, in this context often called a chicken foot.

this would have to be happening in a substantial fraction of the cellular replication forks under normal growth conditions [40,44]. This was the first indication that bacterial replication forks underwent a frequent demise, and that restart was a critical cellular function even in the absence of SOS-inducing conditions. This eventually led Sandler and Marians to rename this protein complex the restart primosome [45].

In bacteria at least, it appears that the nonmutagenic pathways for repairing broken replication forks are more important under most conditions than are the pathways for mutagenic translesion bypass. The major TLS pathway mediated by DNA polymerase V is induced only late in the SOS response, as a sort of last ditch effort to get replication restarted [3,46,47]. DNA polymerase IV, the other TLS polymerase in *E. coli*, has a more cryptic function [47] that may be related to the generation of adaptive mutations in stationary phase cells [48]. The nonmutagenic pathways, in contrast, are in operation at all times and are enhanced by the induction of key proteins at early stages in the SOS response.

## 2. Recombinational DNA repair of replication forks in bacteria

Some of the major pathways for recombinational repair of replication forks are outlined in Fig. 4. The pathways are complex and flexible, reflecting the potentially myriad structures that a cell could be presented with at a broken fork. Two major paths can be envisioned and are experimentally substantiated to some degree.

First, the replication fork can encounter a strand break, resulting in a double strand break that severs one arm of the replication fork (path A in Fig. 4). Fork collapse ensues, and the structure generated is one that cannot be rectified by translesion synthesis. The severed DNA branch is processed by the bacterial RecBCD helicase–nuclease, followed by DNA strand invasion mediated by the RecA protein. Additional processing of the recombination intermediates generates a viable replication fork structure. This branched fork is then reactivated by the restart primosome, which loads DNA polymerase III and the other replication enzymes to restart DNA synthesis.

Notably, in bacterial strains lacking RecBCD function, about 20% of the cells have unrepaired chromosomal double strand breaks under normal growth conditions [49].

Second, the fork can be arrested at the site of a DNA lesion. The possibilities here are quite varied, and only a few are shown in path B in Fig. 4. In the pathway shown, a leading strand lesion has led to fork arrest, leaving a long single strand gap on the leading strand. This arrested fork structure is one of the few that have been documented experimentally [50]. Fork regression to generate a chicken foot is a likely first step in the repair of arrested forks. The chicken foot can be cleaved by a Holliday junction resolvase such as the bacterial RuvC protein, funneling the DNA into the double strand break pathway. Alternatively, the single strand gap (now at the end of the short arm in the chicken foot structure) can be filled in by polymerase, followed by a reverse branch migration to regenerate a fork-like structure. The reversal may happen either before or after repair of the lesion itself, and in either case the lesion is effectively bypassed without introducing a mutation. As a final alternative, the short arm of the chicken foot may be degraded by nuclease, which also generates a fork-like structure. In this final case, the lesion must be repaired before replication can again commence.

The conversion of an arrested fork into a double strand break via cleavage deserves more comment. There is much evidence now that the chicken foot structure can be cleaved, and that stalled forks result in increased numbers of double strand breaks [14,51–54]. The generation of these broken ends at the sites of fork stalling is one factor that can lead to elevated levels of inappropriate types of recombination, which in turn can be a significant contributor to genome instability if not controlled [51,55].

The fork repair pathways are elaborate, and in some cases redundant. The steps can be varied to accommodate almost any conceivable structure at an arrested fork. In the case of fork collapse at a double strand break, the recombination steps actually repair the lesion itself. In the case of fork arrest at a base lesion, the recombination steps merely reconstitute the conditions needed for lesion repair, providing an undamaged complementary strand opposite the strand with the lesion to guide nonmutagenic repair.

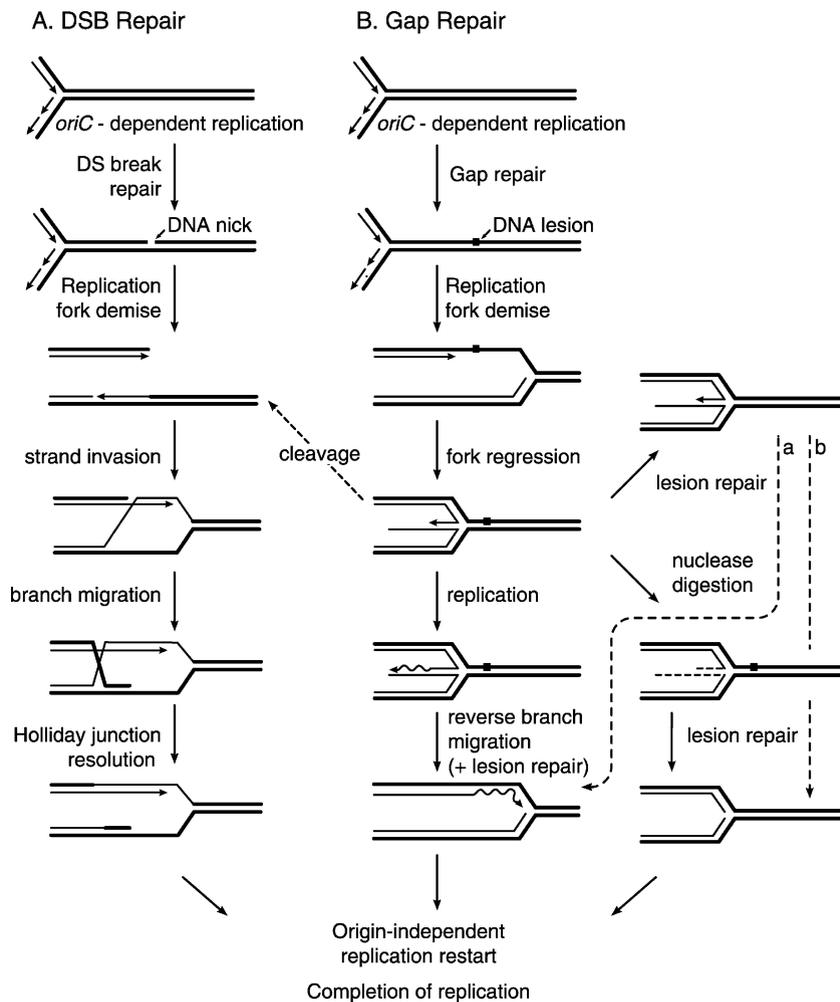


Fig. 4. Some pathways for the nonmutagenic recombinational DNA repair of broken replication forks. Most details are described in the text. The template and newly synthesized DNA strands are indicated by thick and thin lines, respectively. Dashed line pathways a and b denote the processing of a chicken foot after the arresting lesion has been repaired, via either (a) replication and reverse branch migration, or (b) nuclease digestion of the short arm of the chicken foot.

### 3. Enzymes

One can readily list 25 different bacterial proteins directly involved in key steps of recombinational DNA repair of replication forks, excluding the DNA polymerases (Table 1). There are many more involved in auxiliary roles, or roles yet to be elucidated. Table 1 lists some of the key protein classes, with representatives from bacteria, eukaryotes, and the important bacteriophage T4 system.

Recombinases play a major role in replication fork repair. In bacteria, the RecA protein [11,56] promotes DNA strand invasion, and could in principle promote replication fork regression and the subsequent reversal of the chicken foot to regenerate the fork [11,56]. DNA helicases also play potential roles in multiple steps, especially steps involving branch migration. There are at least three potential paths for fork regression. Regression can be promoted by RecA protein [56], although the RecG helicase may be more important to

Table 1  
Enzymes involved in the nonmutagenic repair of broken replication forks

	Bacteriophage T4	<i>E. coli</i>	Eukaryotes
Recombinase	UvsX	RecA	Rad51/Rad54, Dmcl
Single strand binding protein	Gene 32	SSB	RPA
RMP (Rec)	UvsY	RecOR, RecBCD	Rad52, Rad55/Rad57
Branch helicase	UvsW	RecG, RuvAB	
End processing	gp46/gp47	SbcCD, RecBCD	Rad50, Mre11, Xrs2/Nbs1
HJ resolution	Endonuclease VII	RuvC/Rus	
Replication restart RMP	gp59	PriA, restart primosome	
Replicative helicase	gp41	DnaB	
Polymerase	T4 Polymerase	DNA polymerases II/III	Polymerases $\alpha$ , $\delta$ , $\epsilon$ , RFC, PCNA, etc.

the regression process in most fork structural contexts [57,58]. In principle, fork regression can also occur spontaneously, driven by the positive supercoiling built up ahead of the fork by the replication process [29].

The role of recombination/replication mediator proteins (RMPs) may prove to be especially interesting. In bacteria, the RecF, RecO, and RecR proteins have demonstrated roles in mediating the assembly and disassembly of RecA filaments [59–62]. However, it is unlikely that their functions have been fully elucidated. The RecF and RecR proteins are encoded by genes linked to operons encoding subunits of DNA polymerase III and other replication proteins, and speculation has been published that the proteins might be part of an interface function mediating the transition between replication and recombination systems during the repair process [63].

An important new trend is the establishment of *in vitro* systems of greater complexity that model multiple steps in the proposed fork repair pathways. Progress is especially evident in the double strand break repair pathways. Kowalczykowski and coworkers have reconstituted the steps leading up to strand invasion. Beginning with a linear duplex DNA, the ends are processed by the RecBCD enzyme, followed by RecA-mediated DNA strand invasion. Establishment of a complete replication fork requires the restart primosome and DNA polymerase III.

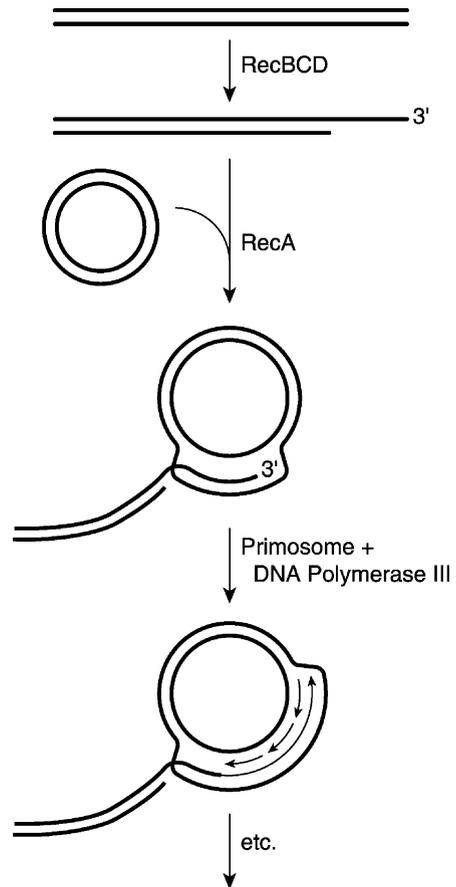


Fig. 5. An *in vitro* system mimicking key steps in the recombinational repair of a collapsed replication fork, developed by Marians and coworkers (see text). The linear duplex DNA is processed by the RecBCD enzyme, followed by RecA-mediated DNA strand invasion. Establishment of a complete replication fork requires the restart primosome and DNA polymerase III.

oriented so that most can intercept RecBCD enzymes traveling away from forks and back toward the replication origin, as they must do to effect fork repair [68]. The processing of the DNA ends is coupled to a RecBCD-facilitated loading of RecA protein onto the extension, preparing it for strand invasion [64–66]. This effort has been extended significantly by Marians and coworkers, who have reconstituted *in vitro* a system in which a duplex DNA is processed by RecBCD, RecA promotes D-loop formation, and a complete replication fork is initiated at the D-loop via the combined action of the restart primosome and DNA polymerase III [69] (Marians, personal communication) (Fig. 5).

#### 4. Questions

Because of its relatively recent recognition as a major DNA repair pathway, the recombinational DNA repair of broken replication forks may be the least well-characterized process in DNA metabolism. Some of the major questions have been reviewed elsewhere [6]. In brief, we have quite limited information on the actual frequencies with which fork-halting lesions occur in cells, and on the structures of replication forks after they are halted. The fate of replication fork enzymes has not been explored, and it is not clear if all of them are always displaced during recombinational repair. Much work remains to find all of the protein components of these systems. This work is well-advanced in bacteria, but many important proteins remain to be identified in eukaryotes (as evidenced in part by the blanks in Table 1). The reconstitution of *in vitro* systems mimicking these repair paths is still at a very early stage of development.

A particularly complex question concerns the coordination of fork repair with other aspects of DNA metabolism. In bacteria, evidence is building that replication forks are organized in “factories” that are anchored to the inner cell membrane [70,71]. This places some potentially interesting spatial and structural constraints on the fork repair processes, and may infer the involvement of organizational proteins yet undiscovered. Part of this organization could involve bacterial homologues of the eukaryotic SMC proteins, such as the SbcCD proteins [21,72] and the RecN

protein [6]. The SbcCD complex has some nuclease activity, but both SbcCD and RecN might play a role in sequestering DNA ends in a tight complex where they could participate readily in appropriate DNA repair processes. In eukaryotes, it is likely that recombinational DNA repair of replication forks is coordinated with cellular checkpoint systems, as outlined below.

Increasingly, insights relevant to the composition of recombinational DNA repair systems and their cellular functioning may come from genomics. Patterns of recombination-facilitating sequences are readily identified in properly organized genomic searches [67,73], as are clusters of genes with repair functions [74]. The methodology now associated with genomics and proteomics should allow expanded efforts to find new proteins that interact with major recombination functions and which potentially participate in recombinational repair of replication forks.

#### 5. Nonmutagenic repair of replication forks in eukaryotes

Elaborate systems for the repair of broken replication forks exist in all eukaryotic cells. It has been difficult to judge their relative importance in the presence of other strong replication and recombination signals. It has been suggested that about 10 replication forks collapse or arrest in a human cell replication cycle [24], although the basis of the estimate has not been detailed. The recent paradigm shift in bacteria, however, has encouraged greater efforts to examine eukaryotic recombination functions from the standpoint of replication repair. The overall theme of recombinational DNA repair of replication forks has rapidly become an organizing principle, providing a compelling working hypothesis that can readily explain a wide variety of experimental observations. A sampling of these observations and their interpretations are summarized here.

##### 5.1. Preferential use of sister chromatids in repair

When replication forks encounter blocking lesions in human DNA, a nonmutagenic pathway for their repair exists that involves the use of sister chromatid recombination [75].

### 5.2. Break-induced replication

In eukaryotes, an origin-independent mode of replication occurs that is analogous to the recombination-dependent recombination seen in bacteriophage T4 and in bacteria. Break-induced replication (BIR) is, in effect, the eukaryotic RDR. The process has been studied most carefully in budding yeast [25]. BIR is a replication event [76] manifested by mitotic non-reciprocal recombination events which can extend for hundreds of kilobase pairs along a chromosome [77–80]. There are at least two pathways of BIR in yeast, and recombination functions are essential to initiate replication in these events [25].

In the absence of telomerase, BIR also provides an avenue for the restoration of a lost chromosomal telomere. The broken chromosome undergoes strand invasion into a homologous chromosome still retaining a telomere, and the resulting replication extends all the way to the end of the second chromosome's telomere [81]. The existence of eukaryotic BIR proves at least that the basic strand invasion activity needed to repair collapsed replication forks, along with the replication restart complex, is present.

### 5.3. The checkpoint connection

When DNA damage occurs in eukaryotes, the cell cycle is blocked or slowed to allow time to repair the damage. The cell cycle control is mediated by a set of DNA damage checkpoints [82–84]. Separate checkpoints exist for different phases of the cell cycle, often with distinct signaling components. The G1 and G2 checkpoints slow the cell cycle to prevent entry into S phase replication or mitosis, respectively, with a damaged chromosome. The S phase checkpoint is distinct, in that a lack of functions specific to this checkpoint (but not those involved in G1, G2, or M checkpoints) give rise to chromosome instability, leading usually to deletions of chromosome ends [85–87]. This result provides a compelling link between replication and recombination, implying that replication problems lead to genomic instability and that the S phase checkpoint suppresses that instability. When replication is compromised by DNA damage, the S phase checkpoint controls replication initiation at late-firing origins [88–91]. Recent evidence indicates that an additional S phase checkpoint pathway exists, a

multi-branched checkpoint called intra S that serves to slow progression through S phase when DNA damage is present [92,93]. Defects in the intra S checkpoint pathways can also produce genome instability. Many of the mutations in S phase checkpoint functions act synergistically, with certain combinations producing 12,000–14,000-fold increases in genome rearrangements [87]. The observed rearrangements in yeast cell genomes provide a good model for the types of genome rearrangements observed in higher eukaryotes in certain types of tumors, and suggest that the S phase checkpoints serve to suppress these rearrangements.

The S phase checkpoint [94] appears to be activated only when replication forks encounter DNA damage [95]. Replication forks collapse irreversibly at a high rate in yeast S phase checkpoint mutants (*mec1* or *rad53*) [96]. In *mec1* mutant cells, fork stalling occurs even in the absence of DNA damaging agents, and double strand breaks arise later in the G2 phase [97]. The forks generally stall, and the breaks are later concentrated within regions called replication slow zones [97]. Unusual DNA structures accumulate if *rad53* mutant yeast cells are treated with hydroxyurea (HU; an agent that blocks replication by depleting nucleotide pools), suggesting that Rad53 may play a role in the stabilization of broken replication forks [98]. Interestingly, the structures that accumulate include stalled forks with extensive single strand gaps, as well as chicken feet [99]. The results provide some direct in vivo observations consistent with postulated pathways of replication fork repair, to substantiate models (Fig. 4), indirect evidence [14], and previous accidental observations of relevant replication intermediates [7].

### 5.4. Recovery from replication blocks and RecQ family helicases

S phase replication can be reversibly arrested in eukaryotes by treatments with agents that block replication or cause DNA damage. The enzymatic basis of the recovery of replication has been explored, with the discovery that RecQ family helicases play a key role the recovery from arrest to complement roles in other aspects of the eukaryotic replication cycle. These studies also provide numerous links between recombination and replication in eukaryotes. RecQ helicases were discovered in bacteria as components of the RecF

recombination pathway [100], and are implicated in a variety of recombination reactions although their precise molecular role has not been clarified. Enzymes in this family include the SGS1 protein of yeast, the rql1 protein of fission yeast, the WRN and BLM helicases in mammals, and the bacterial RecQ helicases. The loss of RecQ family helicase function has been associated in eukaryotes with aberrant replication along with genome instability and an increase in illegitimate recombination. The loss of *rql1* function in *Schizosaccharomyces pombe* leads to defective recovery from the replication arrest caused by treatment with hydroxyurea or UV [101]. Sensitivity to these agents has become a hallmark of null mutants of RecQ family genes in eukaryotes (HU sensitivity has not yet been carefully explored in bacteria). Loss of the function of a WRN ortholog in *Xenopus laevis* prevents the formation of replication foci [102]. The WRN helicase has been shown to interact with a number of replication proteins, including replication protein A and DNA polymerase  $\delta$  [103–105], with the latter interaction specifically facilitating the replication of DNA sequences that tend to form unusual secondary structures [106]. In human cells, loss of WRN function prevents normal recovery from hydroxyurea-induced replication arrest, and an increase in apoptosis [107]. In yeast, RecQ helicases have a number of roles in the replication cycle, including the suppression of hyperrecombination associated with replication inhibition, a role in the S phase replication checkpoint, and the promotion of proper chromosomal segregation after replication [101,108–112]. The yeast SGS1 helicase is involved in the suppression of genomic instability [113].

Thus, it has been proposed that one role of the RecQ proteins is to maintain genomic integrity by suppressing illegitimate recombination and facilitate replication through structural barriers in the DNA [114–117]. This could involve some role in the repair of stalled replication forks, and/or the resolution of aberrant structures in the path of replication forks so that forks stall less often [114–117].

### 5.5. Organization at the replication fork and the SMC proteins

Another manifestation of the recombination/replication connection may be seen in studies of SMC proteins, a class including cohesins and condensins as

well as a range of proteins (e.g. Rad50 in eukaryotes and RecN in bacteria) involved in non-homologous end joining and other recombination and checkpoint processes [118–120] linked to S phase replication. In fission yeast, the loss of Rad50 function results in sensitivity to DNA damage, and an increase in homologous recombination not involving sister chromatids [121]. An interesting possibility is that one role of Rad50 is to keep sister chromatids together during replication, facilitating accurate recombinational repair of replication forks when needed.

The exploration of nonmutagenic repair of replication forks in eukaryotes is expanding, and will be enhanced by the use of genomic methods and the further investigation of the biochemical properties of the proteins involved as they are discovered.

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